

(NE)
In the Claims

Amend claims 1 and 7 as follows.

1. (Amended) Nucleic acid coding for a polypeptide having an amino acid sequence as shown in Fig. 4 or a functional variant thereof, and parts thereof having at least 8 nucleotides, except a nucleic acid having the sequence (SEQ ID NO: 6):

1 GCCAACACGC ANTCCGACGA CAGTGCAGCC ATGGTCATTG CAGAGATGCN TCAAACTCAA
61 TGAGCACATC ACCAACGTAA ACGTCGAGTC CAACTTCATA ACGGSAAGG GGATCCTGSC
121 CATCATGAGA GCTCTCCAGC ACAACACGGT GCTCACGGAG CTGCGTTTCC ATAACDAGAG
181 GCACATCATG GGCAGCCAGG TGGAAATGGA GATTGTCAAG CTNCTGAAGG AGAACACGAC
241 GCTNCTGAGG CTGGGNTACC ATTTTNAACT CCCAGGACC

7. (Amended) Polypeptide having an amino acid sequence as shown in Fig. 4 or a functional variant thereof, and parts thereof having at least 6 amino acids, except a polypeptide having the sequence (SEQ ID NO: 7):

PTRNPTTVQPWSLQRCIKVNEHITNVNVESNFITGKGILAIMRALQ
10 20 30 40
HNTVLTELRFHNQRHIMGSQVEMEIVKLLKENTTLLRLGYHFKLPG
50 60 70 80 90

REMARKS

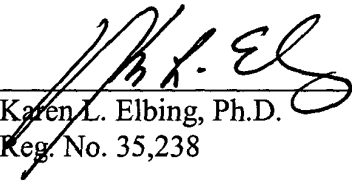
The specification has been amended to provide a unique sequence identification number for each nucleotide sequence within the specification. The attached sequence listing has also been inserted into the application. No new matter is introduced by any of these amendments.

If there are any other charges, or any credits, please apply them to Deposit Account No.

03-2095.

Respectfully submitted,

Date: 16 October 2001



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21559

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Marion Elke Hofmann et al.	Art Unit:	1653
Serial No.:	09/445,362	Examiner:	Carlson, K.
Filed:	May 15, 2000	Customer No.:	21559
Title:	MYOCARDIUM-AND SKELETAL MUSCLE-SPECIFIC NUCLEIC ACID, ITS PREPARATION AND USE		

Assistant Commissioner For Patents
Washington, DC 20231

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

A marked up version of the third full paragraph on page 3 of the specification is presented below.

One aspect of the invention is therefore a nucleic acid coding for a polypeptide having an amino acid sequence as shown in Fig. 4 or a functional variant thereof, and parts thereof having at least 8 nucleotides, preferably at least 10 nucleotides, in particular at least 15 nucleotides, especially at least 20 nucleotides, except a nucleic acid having the sequence (SEQ ID NO: 6):

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1  GCCAACACGC ANTCCGACGA CAGTGCAGCC ATGGTCATTG CAGAGATGCN TCAAAGTCAA
61  TGAGCACATC ACCAACGTAA ACGTCGAGTC CAACTTCATA ACGGGAAAGG GGATCCTGGC
121 CATCATGAGA GCTCTCCAGC ACAACACGGT GCTCACGGAG CTGCGTTTCC ATAACCAGAG
181 GCACATCATG GGCAGCCAGG TGGAAATGGA GATTGTCAAG CTNCTGAAGG AGAACACGAC
241 GCTNCTGAGG CTGGGNTACC ATTTTNAACT CCCAGGACC
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in which N denotes A, T, G or C.

A marked up version of the last paragraph on page 3 and ending on page 4 of the specification is presented below.

The nucleic acid according to the invention was isolated from a human cardiac tissue cDNA bank and sequenced. For this, firstly complete RNA was isolated by standard methods from a healthy and insufficient cardiac sample and transcribed with the aid of a 3' anchor primer mixture, for example a 5'-T₁₂ACN-3' primer, in which N denotes any deoxyribonucleotide, and reverse transcriptase into c-DNA. The cDNA was then amplified with a method based on the so-

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called differential display method of Liang and Pardee (Liang, P. & Pardee, A. (1992) *Science* 257, 967-970) under specific PCR conditions with the aid of a 3' primer, for example a T₁₂ACN primer, and of an arbitrarily selected 5'-decamer primer, for example a 5'-CCTTCTACCC-3' decamer primer (SEQ ID NO:5). It was possible thereby to amplify a 321 base pair (bp)-long DNA fragment which is surprisingly present not in the healthy heart sample but distinctly in the insufficient heart sample. This was surprising because the conventional methods such as the differential display method or else subtractive cDNA gene banks are associated with the problem of redundancy, of underrepresentation and of false-positive clones. In particular, it is possible to identify the gene products of weakly expressed genes only under special conditions. It is therefore also not astonishing that the hit rate is generally very low (10-20%) and, for example in the differential display method, also depends on the chosen PCR conditions, the primer length or, for example in the production of subtractive banks, on the hybridization temperature. The complete gene was then isolated from a cDNA gene bank with the aid of the found DNA fragment and sequenced.

A marked up version of the second full paragraph on page 13 of the specification is presented below.

The present invention further relates to the polypeptide itself having an amino acid sequence as shown in Fig. 4 or a functional variant thereof, and parts thereof having at least 6 amino acids, preferably having at least 12 amino acids, in particular having at least 15 amino acids and especially having at least 164 amino acids, except a polypeptide having the sequence (SEQ ID NO: 7):

PTRNPTTVQPWSLQRCIKVNEHITNVNVESNFITGKGILAIMRALQ				
10	20	30	40	
HNTVLTELRFHNQRHIMGSQVEMEIVKLLKENTTLRLGYHFKLPG				
50	60	70	80	90

A marked up version of the third, fourth, and fifth paragraphs on page 21 in the specification are presented below.

Fig. 1 shows a 1936 nucleotide-long heart-specific DNA sequence (SEQ ID NO: 1). The region which codes for the corresponding polypeptide is shown in bold. The DNA fragment for Example 1 is underlined.

Fig. 2 shows a 2080 nucleotide-long heart-specific DNA sequence which has an extension at the 5' end of the DNA sequence from Fig. 1 (SEQ ID NO: 2). The region which codes for the corresponding polypeptide is once again shown in bold.

Fig. 3 shows a 2268 nucleotide-long heart-specific DNA sequence which has an extension at the 5' end of the DNA sequence from Fig. 1 or Fig. 2 (SEQ ID NO: 3). The

A marked up version of the first full paragraph on page 22 in the specification is presented below:

Fig. 4 shows a 552 amino acid-long polypeptide sequence encoded by one of the DNA sequences shown in Figs. 1-3 (SEQ ID NO: 4). The regions homologous with human tropomodulin are shown in bold. The sequence motifs which indicate regulation of the polypeptide by tyrosine kinase signal transduction pathways are underlined.

A marked up version of the third full paragraph on page 22, ending on page 23 of the specification is presented below.

Complete RNA was initially isolated by standard methods (Chomczynski & Sacchi (1987), *Anal. Biochem*, 162 (1), 156-159) from a healthy and an insufficient cardiac tissue sample. The RNA was then treated with DNase in order to remove DNA contamination. An aliquot of this RNA (0.2 µg) was then incubated in a 20 µl reaction mix with 1 × RT buffer (Gibco Y00121), 10 mM DTT, 20 µM dNTP mix, 1 U/µl RNasin (Promega N2511), 1 µM 3' anchor primer mixture of the 5'-T₁₂ACN-3' type, where N can be any deoxynucleotide, and 10 U/µl SuperScript RNase H⁻ reverser transcriptase at 37°C for 60 min and thus transcribed into cDNA. A cDNA aliquot was then subjected to a 20 µl PCR in 1 × PCR buffer (Perkin-Elmer) which, besides 1 µM 3' primer T₁₂AC and 1 µM 5'-decamer primer (5'-CCTTCTACCC-3') (SEQ ID NO: 5), contains 10 µCi of α-³²P-dCTP, 2 µM dNTP mix and 1 U of AmpliTaq (Perkin Elmer). The mixture was incubated firstly at 94°C for 1 min, then 40 cycles each of 30 s at 94°C, 40°C for 2 min and 72°C for 30 s and finally at 72°C for 10 min. The resulting DNA fragment mixture was then fractionated on a 6% polyacrylamide gel and autoradiographed. A DNA fragment which is 321 bp in length and which is not present in the healthy heart sample but is distinctly present in the insufficient heart sample is thus prepared. This fragment was then cut out of the gel on the basis of the X-ray film and was reamplified by PCR under the conditions already described. The resulting fragment was then cloned into an appropriate vector, and the DNA sequence was determined. A fragment prepared in this way contains nucleotides 1627-1936 of the sequence according to Claim 1 and the 12 thymine nucleotides from the 3' anchor primer.

In the Claims:

1. (Amended) Nucleic acid coding for a polypeptide having an amino acid sequence as shown in Fig. 4 or a functional variant thereof, and parts thereof having at least 8 nucleotides, except a nucleic acid having the sequence (SEQ ID NO: 6):

7. (Amended) Polypeptide having an amino acid sequence as shown in Fig. 4 or a functional variant thereof, and parts thereof having at least 6 amino acids, except a polypeptide having the sequence (SEQ ID NO: 7):